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## Appendix 5: Foraging Behavior with Radio Frequency Identification

## **CONTENTS**

A5.1	Experimental Procedure	95
	TO C	96

Experimental test situations have been designed to explore feeding behavior and social communication (Schricker and Stephen, 1970; Cox and Wilson, 1984; Bortolotti et al., 2003; Yang et al., 2008). These studies generate information on trips between a feeder and a hive, with the variable of pesticide exposure explored. Most test techniques (in this area of exploration) are limited by the number of individuals that can be simultaneously monitored, and by the time devoted to recording individuals. To address these limitations, automated tracking and identification systems have been developed using radio frequency (RF) transponder technology. The use of transponders has the potential to revolutionize the study of insect life-history traits, especially in behavioral ecotoxicology.

Different transponder devices have been employed on the honey bees, including harmonic radar (e.g., Riley and Smith, 2002) and radio frequency identification (RFID Streit et al., 2003). Currently, the RFID tags seem to offer unique advantages. Advantages of the RFID technology include the large number of individual insects that can be tracked, the number of detections that can be monitored rapidly and simultaneously (milliseconds) without interference from a variety of matrices (e.g., propolis, glue, plastic, wood) which frequently encumber visual observations. RFID is also less disruptive on bee behavior given the small size of the tags compared to what is needed for harmonic radar tracking.

The tag itself is not equipped with a power source (passive function); rather, it obtains its signal power from the detector (transponder) and causes the tag to emit a unique identification code. The detector (reader) can recognize a virtually unlimited number ( $18 \times 10^{18}$  possible identification codes) of individually tagged insects. The RFID technology allows detecting each time a tag-equipped bee is passing in close proximity to the reader (working distance of approximately 3 m). In a study to determine the error rate, Streit et al., 2003 demonstrated that 1 out of 300 tagged bees was not recorded by the RFID readers.

## A5.1 EXPERIMENTAL PROCEDURE

The experimental colony is maintained in an outdoor tunnel (8 m  $\times$  20 m, 3.5 m high) covered with an insect-proof cloth and the ground covered with a double layer of plastic. Bees are fed with pollen that is renewed daily. A sucrose solution (50% w/w) is delivered by a feeder positioned 18 m from the hive entrance, in a wooden box (26  $\times$  26 cm, 30 cm high).

RFID tags (64-bit, 13.56 MHz system,  $1.0 \times 1.6 \times 0.5$  mm), weighing about 3 mg (3% of bees' weight), represent a relatively low weight given that the honey bee is able to carry up 70 mg of nectar (Ribbands, 1953) and 10 mg of pollen (Hodges, 1952). A tag-equipped bee passing underneath the reader is identified by

Pesticide Risk Assessment for Pollinators, First Edition. Edited by David Fischer and Thomas Moriarty. © 2014 John Wiley & Sons, Inc. Published 2014 by John Wiley & Sons, Inc.

196

the reader that sends the data along with real-time recording to a database. Readers are placed at the entrance of the hive and at the artificial feeder. By passing underneath the reader, both at the hive and at the feeder, the foraging bee is monitored twice, thus determining the direction of travel and the travel time between the two recording points. The reader software records the identification code and the exact time of the detection automatically for 6 days in a database for later analysis of spatial and temporal information. Analyses of the data may provide information on the time spent within the hive; time spent at the feeder; time spent between the feeder and the hive; the number of entries into and exits from the hive; and the number of entries into and exits from the feeder.

RFID devices allow the study of both the behavioral traits and the lifespan of bees, especially under biotic and/or abiotic stress. However, the large quantity of data obtained with this technique requires an interface for analyzing the data and providing the life-history traits of individual bees. Under semi-field conditions, RFID microchips have provided detectable effects due to exposure to an insecticide (Decourtye et al., 2011).

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